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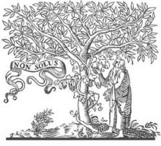

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Neuropharmacology and analgesia

The use of carbetapentane for spinal anesthesia and use-dependent block of sodium currents

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ABSTRACT

Although carbetapentane produces skin (peripheral) infiltrative analgesia, the underlying mechanism of carbetapentane in local anesthesia is not well understood. The purpose of the study was to examine the effect of carbetapentane on voltage-gated Na^+ channels and its efficacy on spinal (central) anesthesia. We evaluated the effects of carbetapentane on rat motor and pain behavior (when administered intrathecally) and on voltage-gated sodium channels in differentiated neuronal NG108-15 cells. Carbetapentane exhibited dose-dependent spinal blockade with a more sensory-selective action over motor blockade ($P<0.05$). Carbetapentane was more potent than lidocaine ($P<0.05$) in spinal anesthesia. Intrathecal 5% dextrose (vehicle) elicited no spinal anesthesia. Lidocaine, used as a positive control, demonstrated concentration- and state-dependent effects on tonic block of voltage-gated Na^+ currents (IC_{50} of 49.6 and 194.6 μM at holding potentials of −70 and −100 mV, respectively). Carbetapentane was more potent (IC_{50} of 36.3 and 62.2 μM at holding potentials of −70 and −100 mV, respectively). Carbetapentane showed a much stronger frequency-dependence of block than lidocaine: with high frequency stimulation (3.33 Hz), 50 μM lidocaine produced an additional 30% blockade, while the same concentration of carbetapentane produced 70% more block. These results revealed carbetapentane had a more potent and prolonged spinal blockade with a more sensory/nociceptive-selective action over motor blockade in comparison with lidocaine. Spinal anesthesia with carbetapentane could be through inhibition of voltage-gated Na^+ currents.

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1. Introduction

Carbetapentane, a known non-opioid antitussive, produces typical atropine-like effects and is used effectively in the treatment of acute cough owing to infections of the upper respiratory tract (Donmez et al., 2011). Recently, it has been shown that continuing intravenous administration of equipotent carbetapentane is less toxic than bupivacaine to the central nervous system and cardiovascular system (Hung et al., 2012). Furthermore, block duration of carbetapentane was similar to bupivacaine in providing infiltrative cutaneous (peripheral) local anesthesia (Hung et al., 2012). Spinal (central) anesthesia is known to be a relatively simple method, which supports suitable surgical conditions through the injection of a small amount of local anesthetic with easy landmarks (Hung et al., 2009; Vandermeersch et al., 1991). To the best of our knowledge, spinal anesthesia with carbetapentane has not yet been reported.

Inhibition of voltage-gated Na^+ currents, which is one of the major mechanisms of local anesthesia, elicits spinal anesthesia, skin infiltration anesthesia, and sciatic nerve block (Borgeat and Aguirre, 2010; Vegh et al., 2006). Furthermore, White et al. reported that both carbetapentane and a potent local anesthetic dibucaine decreased action potential (AP) discharge using whole cell current-clamp recording from CA1 pyramidal neurons (White et al., 2012). However, no reports are available about the effects of carbetapentane on voltage-gated Na^+ channels to date. The aim of this study was to evaluate whether carbetapentane produced spinal anesthesia and blocked Na^+ currents. In *in vivo* studies, we examined dose-related spinal blockade of motor function,

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proprioception, and nociception following intrathecal injection of carbetapentane; in *in vitro* studies, we assessed whether carbetapentane could suppress Na^+ currents using the patch-clamp method. Lidocaine, a traditional local anesthetic, was used as a control.

2. Materials and methods

2.1. Part 1—in vivo experiments

2.1.1. Animals

Male adult Sprague-Dawley rats (295–345 g) were purchased from the National Laboratory Animal Centre (Taipei, Taiwan), and then were housed in groups of three, with food and water freely available until the time of experiments. The temperature-controlled room was maintained at 22 °C with approximately 50% relative humidity on a 12-h light/dark cycle (6:00 a.m.–6:00 p.m.). The experimental protocol was approved by the Institutional Animal Care and Use Committee of China Medical University, Taiwan, and conformed to the recommendations and policies of the International Association for the Study of Pain (IASP).

2.1.2. Drugs

Carbetapentane citrate and lidocaine HCl monohydrate were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Before intrathecal injection, all drugs were freshly prepared in 5% dextrose as solution.

2.1.3. Intrathecal injection

Spinal block through intrathecal drug injection was performed on conscious rats as previously described (Leung et al., 2012, 2010). All animals were injected once in this study. Before intrathecal injection, each 50-μl of 0.5% lidocaine was injected into the right- and left-side of paraspinal space (0.5 cm in depth) which was 0.5 cm away from the mid-point of the longitudinal line of the lumbar 4–5 (L4–L5) intervertebral space. Five minutes later, a 27-gauge needle attached to a 50-μl syringe (Hamilton, Reno, Nevada) was inserted into the mid-line of the L4–L5 intervertebral space and 50-μl of drugs (carbetapentane and lidocaine) or vehicle (5% dextrose) was injected. The animals were observed for the development of spinal anesthesia, as indicated by paralysis of both hind limbs.

2.1.4. Neurobehavioral measurements

Three neurobehavioral examinations, which consisted of evaluations of proprioception, nociception, and motor function was conducted after intrathecal injection (Chen et al., 2012b, 2012c). The magnitude of spinal blockade in proprioception, nociception, and motor function was described as the percent of possible effect (% PE). The maximum block in a time course of spinal anesthesia with drugs was described as the percentage of maximal possible effect (% MPE). Animals were evaluated before medication and at 1, 3, 5, 7, 10, 15, and 20 min afterwards, then again at 10-min interval until 1 h, at 15 min interval until 2 h and at 30 min interval until 5 h. For consistency, a trained investigator, who was blinded to the identity of the injected drugs, was responsible for handling of all rats and behavioral assessments.

Proprioception was based on the resting posture (‘tactile placing’ and ‘hopping’) and postural reactions. Hopping response was tested by lifting the front half of the animal off the ground and lifting one hind limb at a time off the ground so that the animal was standing on just one limb. Then, the animal was moved laterally, which normally evoked a prompt hopping response with the weight-bearing limb in the direction of movement to prevent the animal from falling. A predominantly motor impairment showed a prompt but weaker than normal response. Conversely, with a predominantly proprioceptive block, delayed hopping was followed by greater lateral hops to prevent falling over or, in this case of complete block, no hopping at all. The functional deficit was quantified as 3 (normal or 0% MPE), 2 (slightly impaired), 1 (severely impaired), and 0 (completely impaired or 100% MPE) (Hung et al., 2011a, 2011b).

Nociceptive reaction was examined by the withdrawal reflex or vocalization exhibited *via* pinching a skin fold over each rat's back at 1 cm from the proximal part of the tail, the lateral metatarsus of bilateral hind limbs, and the dorsal part of the mid-tail. At each testing time, only one pinch was given to each of the four testing sites, and the time interval between stimulations at different sites was around 2 s. The blockade of nociception was quantified as 4 (normal or 0% MPE), 3 (25% MPE), 2 (50% MPE), 1 (75% MPE), and 0 (absent or 100% MPE) (Chen et al., 2012a, 2011b).

Motor function was assessed by measuring ‘the extensor postural thrust' of the right hind limb of the rats. The extensor thrust was measured as the gram force, which resisted contacting the platform *via* the rat heel applied to a digital platform balance (Mettler Toledo, PB 1502-S, Switzerland). The reduction in this force, representing decreased extensor muscle tone, was considered as a deficit of motor function and expressed as a percentage of the control force. The pre-injection control value was considered as 0% motor block or 0% MPE. A force less than 20 g (also referred to as the weight of the ‘flaccid limb’) was interpreted as the absence of extensor postural thrust or a 100% motor block or 100% MPE (Thalhammer et al., 1995). The % PE is calculated *via* the equation

$$\%PE = (Gm - Gp)/(Gm - 20) \times 100\%$$

where Gm is the peak muscle force (*g*) of each rat before *drug injection* and Gp is the peak muscle force (*g*) of each rat after intrathecal injection (Chen et al., 2011a, 2010).

2.1.5. The 50% effective dose (ED_{50}), time to full recovery, and area under curves (AUCs)

The dose–response curves were constructed after intrathecally injecting the rats with 4 different doses of each drug ($n=8$ for each dose of each drug). The curves were then fitted using SAS Nonlinear (NLIN) Procedures (SAS Institute Inc., Carey, NC), and the value of ED_{50}, defined as a dose that elicited 50% spinal blockade, were obtained (Chen et al., 2011c; Minkin and Kundhal, 1999). Time to full recovery, defined as the interval from drug injection to full recovery (0% block or 0% MPE), produced by each drug was recorded. Furthermore, the %MPE, time to full recovery, AUCs of spinal anesthesia with carbetapentane (2 μmol) were compared with those of lidocaine (2.75 μmol). The AUC of spinal blockade of drug was obtained by using Kinetica v 2.0.1 (MicroPharm International, USA).

2.2. Part 2—in vitro experiments

2.2.1. Cell culture

NG108-15 cells were cultured at 37 °C in 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA) and penicillin–streptomycin (100 units/ml, 100 μg/ml) (Invitrogen). NG108-15 cells were induced to differentiate into more mature neurons by being incubated in the above medium with 0.1% fetal bovine serum, 10 μM retinoic acid and 30 μM forskolin for 3 days.

2.2.2. Measurement of voltage-gated Na^+ currents

Electrophysiological measurements were performed as previously described (Leung et al., 2010). Cells were voltage-clamped in the whole-cell mode. Borosilicate glass tubes (OD 1.5 mm, ID 1.10 mm, Sutter Instrument, Novato, CA) were prepared with a micropipette

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